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(54) Title: METHODS OF NUCLEIC ACID AMPLIFICATION

(57) Abstract: The present invention provides a method of simultaneously amplifying a plurality of target sequences within sample nucleic acid which comprises: (a) contacting said sample nucleic acid with one or more primer pairs under conditions which allow hybridisation of the primers to the sample nucleic acid, each primer having a bipartite structure A-B wherein part A is specific for a particular target sequence within the sample nucleic acid and part B is a constant sequence which is common to all primers or is common amongst all forward primers with a different sequence common amongst all reverse primers; (b) performing a first amplification reaction; (c) degrading the bipartite primers or separating them from the amplification products of the first amplification reaction; (d) contacting the amplification products from the first amplification reaction with primers which comprise part B of the bipartite primers or a nucleotide sequence which is substantially identical to part B, under conditions which allow hybridisation of the primers to the amplification products; and (e) performing a second amplification reaction and kits for use in such methods.





### INTERNATIONAL SEARCH REPORT

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A. CLASSII	FICATION OF SUBJECT MATTER C12Q1/68			
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MEDLIN	E, EPO-Internal, BIOSIS, WPI Data, F	^AJ		
C. DOCUME	ENTS CONSIDERED TO BE RELEVANT			
Category •	Citation of document, with indication, where appropriate, of the rel	evant passages		Relevant to claim No.
Х	WO 00 75369 A (REPP REINALD ;RASO WOLFGANG (DE)) 14 December 2000 (2000-12-14) page 1 -page 16	HER		1–26
X	GRACE MARCY B ET AL: "Degradable outer primers in merged tandem (M/T)-nested PCR: Low- and single target amplification" ANALYTICAL BIOCHEMISTRY, ACADEMIC SAN DIEGO, CA, US, vol. 263, no. 1, 1 October 1998 (1998-10-01), page XP002152587 ISSN: 0003-2697 the whole document	e-copy DNA		1-26
X Furth	ner documents are listed in the continuation of box C.	χ Patent family member	ers are listed in	аппех.
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## INTERNATIONAL SEARCH REPORT

PCT/GB 03/00195

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C.(Continu Category °	ation) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
Jalogory -	Onation of Goodings, man indication, miles appropriate, or the relevant passages	Heievan to dam No.		
Х	WO 98 35058 A (RIBOZYME PHARM INC) 13 August 1998 (1998-08-13) the whole document	1-26		
X	HEATH K E ET AL: "Universal primer quantitative fluorescent multiplex (UPQFM) PCR: a method to detect major and minor rearrangements of the low density lipoprotein receptor gene."  JOURNAL OF MEDICAL GENETICS. ENGLAND APR 2000, vol. 37, no. 4, April 2000 (2000-04), pages 272-280, XP001055883 ISSN: 0022-2593 cited in the application the whole document	1-26		
Υ	WO 01 94634 A (BIOPOOL INT INC) 13 December 2001 (2001–12–13) page 1 -page 20; claims 1–26	1-26		
Υ	WO 01 55454 A (GENETRACE SYSTEMS INC; LOEHRLEIN CHRISTINE (US); MONFORTE JOSEPH A) 2 August 2001 (2001-08-02) page 22 -page 34; examples 5,7,8,10,13	1–26		
Y	WO 99 58721 A (WHITEHEAD BIOMEDICAL INST; LANDER ERIC S (US); WANG DAVID G (US)) 18 November 1999 (1999-11-18) cited in the application page 3 -page 9; claims 1,13,27; figures 1,2; example 5	1-26		
Υ	US 5 882 856 A (SHUBER ANTHONY P) 16 March 1999 (1999-03-16) the whole document	1-26		
Y	BROWNIE JANNINE ET AL: "The elimination of primer-dimer accumulation in PCR" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 16, 1997, pages 3235-3241, XP002152588 ISSN: 0305-1048 the whole document	1,5,6		
A	POLZ M F ET AL: "Bias in template-to-product ratios in multitemplate PCR."  APPLIED AND ENVIRONMENTAL MICROBIOLOGY. UNITED STATES OCT 1998, vol. 64, no. 10, October 1998 (1998-10), pages 3724-3730, XP002261301 ISSN: 0099-2240 the whole document	1-26		
	10 (continuation of second sheet) (Juty 1992)			

Form PCT/ISA/210 (continuation of second sheet) (July 1992)



## INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB 03/00195

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0075369 A	14-12-2000	DE AU WO	19925448 A1 5219100 A 0075369 A1	07-12-2000 28-12-2000 14-12-2000
WO 9835058 A	13-08-1998	AU WO	6317398 A 9835058 A2	26-08-1998 13-08-1998
WO 0194634 A	13-12-2001	US AU CA EP WO	6605451 B1 6532701 A 2410281 A1 1352087 A2 0194634 A2	12-08-2003 17-12-2001 13-12-2001 15-10-2003 13-12-2001
WO 0155454 A	02-08-2001	AU CA EP WO US US	3460801 A 2398107 A1 1257664 A1 0155454 A1 2003204322 A1 2002160361 A1	07-08-2001 02-08-2001 20-11-2002 02-08-2001 30-10-2003 31-10-2002
WO 9958721 A	18-11-1999	AU WO	3984699 A 9958721 A1	29-11-1999 18-11-1999
US 5882856 A	16-03-1999	AT AU CA DE EP JP WO US	232242 T 703417 B2 6163696 A 2223729 A1 69626111 D1 0832290 A1 11507226 T 9641012 A1 6207372 B1	15-02-2003 25-03-1999 30-12-1996 19-12-1996 13-03-2003 01-04-1998 29-06-1999 19-12-1996 27-03-2001